Ca$^{2+}$-Induced Ca$^{2+}$ Release Mediates a Slow Post-Spike Hyperpolarization in Rabbit Vagal Afferent Neurons

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Moore, Kimberly A., Akiva S. Cohen, Joseph P. Y. Kao, and Daniel Weinreich. Ca$^{2+}$-induced Ca$^{2+}$ release mediates a slow post-spike hyperpolarization in rabbit vagal afferent neurons. J. Neurophysiol. 79: 688–694, 1998. The relation between Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) elicited by action potentials (APs) and a Ca$^{2+}$-dependent slow post-spike hyperpolarization (AHP$^{\text{slow}}$) in acutely dissociated adult rabbit nodose neurons was studied using microfluorometric calcium measurements in conjunction with standard intracellular current- and voltage-clamp recording techniques. The magnitude of the AP-induced transient increase in [Ca$^{2+}]_{i}$, (ΔCa$^{2+}$) was used to monitor CICR. There was a close correlation between the magnitude of the ΔCa$^{2+}$ and the AHP$^{\text{slow}}$ current over the range of 1–16 APs (r = 0.985). Functional CICR blockers, ryanodine (10 μM), thapsigargin (100 nM), 2,5-di(t-buty1)hydroquinone (10 μM) or cyclopiazonic acid (10 μM), selectively reduced the peak amplitude of the AHP$^{\text{slow}}$ ≥91%. In five neurons, simultaneous recordings of the ΔCa$^{2+}$ and the AHP$^{\text{slow}}$ revealed that both responses were blocked in parallel. These findings indicate that CICR is necessary for the generation of the AHP$^{\text{slow}}$ in rabbit nodose neurons. The ΔCa$^{2+}$ rises and decays significantly faster than the AHP$^{\text{slow}}$. This temporal disparity suggests that activation of the AHP$^{\text{slow}}$ by Ca$^{2+}$ may require additional signal transduction steps.

INTRODUCTION

Afferent information in the peripheral nervous system is encoded by modulation of action potential (AP) frequency. Activation of distinct classes of potassium channels can dramatically affect the frequency and the pattern of neuronal firing. In ~35% of the ~16,000 vagal somata (inferior or nodose ganglion neurons) of the rabbit, the pattern of AP firing can be effectively modified by a Ca$^{2+}$-dependent K$^{+}$ current. This current produces a slowly developing and persistent post-spike hyperpolarization (AHP$^{\text{slow}}$) that plays a significant role in the regulation of membrane excitability, and is responsible for spike frequency accommodation in these neurons (Fowler et al. 1985; Higashi et al. 1984; Weinreich and Wonderlin 1987). Following a single AP, the AHP$^{\text{slow}}$ displays a slow rise time-to-peak (0.3–0.5 s) and a long duration (3–15 s). The critical importance of the AHP$^{\text{slow}}$ in mediating spike frequency accommodation is exemplified by observations that its inhibition by various endogenous autacoids results in an increase in firing frequency from <0.1 to >10 Hz (Undem and Weinreich 1993; Weinreich and Wonderlin 1987). Consistent with a functional role of the AHP$^{\text{slow}}$, some of these autacoids also produce an augmentation in impulse activity recorded in vagal afferents in vivo (Coleridge and Coleridge 1984). The AHP$^{\text{slow}}$ is not restricted to vagal afferent neurons; analogous slow afterpotentials have been characterized in sympathetic, myenteric, and CNS neurons (reviewed by Sah 1996).

Observations of the AHP$^{\text{slow}}$ recorded in rabbit nodose neurons, and analogous data from other nerve cells, suggest that Ca$^{2+}$-discharged from a Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) pool may contribute to the generation of the AHP$^{\text{slow}}$. Intracellular calcium chelation with ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (Higashi et al. 1984) or bis-(o-aminophenoxy)-N,N',N'-tetraacetic acid (BAPTA) (Cohen et al. 1994) abolishes the AHP$^{\text{slow}}$. Manipulations that increase [Ca$^{2+}]_{i}$, such as intracellular injection of Ca$^{2+}$, or applications of ouabain or low concentrations of caffeine, augment and prolong the AHP$^{\text{slow}}$ (Higashi et al. 1987). CICR channel modulators (e.g., ryanodine) or sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) inhibitors [e.g., thapsigargin or 2,5-di(t-buty1)hydroquinone (DBHQ)] block an analogous AHP$^{\text{slow}}$ in sympathetic (Jobling et al. 1993; Kawai and Watanebe 1989, 1991), parasym pathetic (Yoshizaki et al. 1995), and vagal dorsal motor nucleus neurons (Sah and McLachlan 1991).

In rabbit nodose neurons, Ca$^{2+}$ influx produced by 1–7 APs is amplified 5- to 10-fold by CICR (Cohen et al. 1997). The time courses of the CICR-dependent transient increases of intracellular free Ca$^{2+}$ and the AHP$^{\text{slow}}$ appear qualitatively similar (Cohen et al. 1994), suggesting that mobilization of Ca$^{2+}$ from a CICR pool may underlie the AHP$^{\text{slow}}$. In the current work we describe experiments using physiological stimuli, in conjunction with manipulations of CICR, to demonstrate that CICR is essential for the development of the AHP$^{\text{slow}}$.

METHODS

Cell dissociation

New Zealand white rabbits of either sex weighing 1–2 kg were obtained from Robinson Services (Winston-Salem, NC) and killed by pentobarbital sodium overdose (100 mg/kg), as approved by the Institutional Animal Care and Use Committee. Dissociated nodose neurons were prepared as described previously (Leal-Cardoso et al. 1993). After a 3–12-h incubation at 37°C, neurons were maintained at room temperature (22–24°C) to minimize neurite outgrowth and were suitable for experimental use for at least 3–4 days (Magee and Schofield 1991; and our own observations). There were no observable differences in visual appearance or basic electrophysiological properties of the cells based on the sex of the animals or length of time neurons were kept in culture.
Electrode fabrication, recording chamber, and drug delivery

Intracellular recording microelectrodes were fabricated on a Flaming/Brown model P-97 micropipette puller (Sutter Instrument, San Francisco, CA). The aluminosilicate micropipettes (Sutter) had resistances ranging from 30 to 70 MΩ when filled with 4 M potassium acetate or 3 M potassium chloride.

A custom recording chamber provided superfusion (3–5 ml/min) of a 25-mm coverslip with physiological salt solution via a gravity-flow system. It was mounted on the stage of an inverted microscope (Zeiss IM35) equipped with a ×40 phase-contrast oil-immersion objective (Fluor, NA 1.3, Nikon) to allow direct visualization of neurons for intracellular recording and fluorescence measurements. In some experiments where only electrical measurements were made, we used a compound microscope equipped with Hoffman optics (×400).

Electrophysiologic recording

Standard intracellular stimulating and recording techniques were used to monitor electrical activity with “sharp” micropipettes (see Christian et al. 1989 for details). Current-clamp recordings were made with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) either in bridge (filtering at 10 kHz) or in the discontinuous mode (sample rate 5 kHz, filtering at 3 kHz). AP-induced AHP$_{\text{low}}$ currents ($I_{\text{AMP}}$) were recorded using a hybrid voltage-clamp technique. Varying numbers of APs were evoked by transmembrane depolarizing current pulses (3 nA, 3 ms, 10 Hz). One hundred milliseconds after the final depolarizing current pulse, the amplifier was electronically switched from current-clamp to voltage-clamp mode to record the $I_{\text{AMP}}$. Current and voltage signals were digitized with a Neurocorder (Neurodata Instruments, Delaware Water Gap, NJ) for storage on videocassette tapes for offline analysis. The membrane input resistance ($R_m$) of the cell was monitored by measuring the magnitude of the electrotonic voltage transient produced by hyperpolarizing current pulses (100 pA, 150 ms). Analysis of electrophysiologic data were performed using pClamp 6.2 software (Axon Instruments).

During experiments the cells were superfused with 22–24°C Locke solution that had the following composition (in mM): 136 NaCl, 5.6 KCl, 1.2 NaHPO$_4$, 14.3 NaHCO$_3$, 1.2 MgCl$_2$, 2.2 CaCl$_2$, and 10 dextrose (continuously equilibrated with 95% O$_2$/5% CO$_2$; pH 7.2–7.4).

Reagents

Reagents were procured from the following vendors: thapsigargin from LC-laboratories (Woburn, MA), ryanodine and cyclopiazonic acid from Calbiochem (La Jolla, CA), and fura-2/acetoxy-methyl ester (AM), BAPTA/AM, and BAPTA sodium salt from Molecular Probes (Eugene, OR). Inorganic salts were obtained from VWR (Piscataway, NJ).

Unless otherwise noted, drug solutions were prepared daily from concentrated (>10 mM) stock solutions that were stored frozen. Drugs were delivered via the superfusate by switching a three-way valve to a reservoir containing a known concentration of the drug in oxygenated Locke solution.

Ca$^{2+}$ measurements

To measure Ca$^{2+}$, cells on coverslips were incubated for 45–60 min at room temperature (22–24°C) in a solution containing 1 μM fura-2/AM as previously described (Cohen et al. 1994). After incubation the coverslip was placed in the recording chamber and superfused with Locke solution. Fura-2 fluorescence measurements were performed with a DeltaScan Illumination System (Photon Technology International (PTI), South Brunswick, NJ) coupled to the microscope through a fiber optic cable. Each neuron under study was alternately illuminated with 340-nm and 380-nm light and the fluorescence emission, after passing through a 510-nm band-pass filter, was sampled by a photomultiplier tube, the output from which was digitized and stored for subsequent analysis.

Instrument control, data acquisition, and analysis were performed using FELIX 1.1 software (PTI) running on a dedicated microcomputer.

[Ca$^{2+}$], calibration

All fura-2 fluorescence records were corrected for background fluorescence by subtracting the light intensity measured after cell lysis with digitonin. Values of intracellular [Ca$^{2+}$] ([Ca$^{2+}$]) were calculated using the equation of Grynkiewicz et al. (1985)

$$[\text{Ca}^{2+}]_i = K_d \times [(R - R_{\text{min}})/(R_{\text{max}} - R)] \times [(S_0)/(S)]$$

where $R$ is the ratio $F_{340}/F_{380}$, $R_{\text{min}}$ and $R_{\text{max}}$ are the minimum and maximum values of the ratio, attained at zero and saturating Ca$^{2+}$ concentrations, respectively. $F_{340}$ is the fluorescence emitted by the dye when excited at 340 nm, and $F_{380}$ is the fluorescence emitted by the dye when excited at 380 nm. $S_0/\text{S}_i$ is the ratio of fluorescence intensities for Ca$^{2+}$-free and Ca$^{2+}$-bound indicator measured with 380-nm excitation. $R_{\text{min}}$, $R_{\text{max}}$, and $S_0/\text{S}_i$ were determined from six acutely dissociated neurons used specifically for calibration purposes.

Data are expressed as means ± SE. Analysis of variance (ANOVA) and Student’s t-test were used to assess significant differences between calculated means; $P < 0.05$ was considered significant. Unless specified, results were replicated in at least three different neurons.

RESULTS

Effect of BAPTA on the AHP$_{\text{slow}}$

AP-induced elevations of [Ca$^{2+}$], $\Delta$Ca$_i$, recorded in rabbit nodose neurons are strictly dependent on an intact CICR process (Cohen et al. 1997). Additionally, superfusion of acutely isolated rabbit nodose neurons with the AM ester form of the Ca$^{2+}$ chelator BAPTA (25 μM) concomitantly abolishes both the AHP$_{\text{slow}}$ and $\Delta$Ca$_i$ (Cohen et al. 1994). In the current work, we have extended these results and tested whether BAPTA treatment affected Ca$^{2+}$ influx. In 11 neurons incubated with 10–30 μM BAPTA/AM, the AHP$_{\text{slow}}$ was completely blocked within 2–7 min (Fig. 1A). By contrast, incubating neurons with the membrane-impermeant sodium salt of BAPTA (20–30 μM) for 8 min produced no measurable effect on the AHP$_{\text{slow}}$ ($n = 3$). Although the reduction of the AHP$_{\text{low}}$ by intracellular BAPTA is most likely due to Ca$^{2+}$ buffering, BAPTA could conceivably alter Ca$^{2+}$ influx through voltage-dependent calcium channels (VDCCs). However, because the fast Ca$^{2+}$-dependent afterhyperpolarization (AHP$_{\text{fast}}$) following each AP was unaffected by intracellular BAPTA loading, it appears that Ca$^{2+}$ influx through VDCCs is not compromised (cf. Fig. 1, Ba and Bb; $n = 10$). When the superfusate was switched to one containing 100 μM CdCl$_2$, a blocker of Ca$^{2+}$ influx, the AHP$_{\text{fast}}$ was reduced in amplitude (Fig. 1Bc). The small remaining component of the AHP$_{\text{fast}}$ is presumably a reflection of the delayed rectifier. In this neuron, abolition of the AHP$_{\text{slow}}$ was accompanied by a marked increase in the excitability.
FIG. 1. Effect of bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid/acetoxymethyl ester (BAPTA/AM) on slow postspike hyperpolarization (AHP slow) and on excitability in an isolated nodose neuron.

A: AHP slow elicited by 2 action potentials (APs) in control Locke solution and in Locke solution containing 10 μM BAPTA/AM. APs were evoked by transmembrane depolarizing current pulses (4 nA, 1.5 ms, 10 Hz) and are truncated. Dashed line represents the resting membrane potential (−60 mV). Resting membrane input resistance was 70 MΩ. Bath application of BAPTA/AM blocks the AHP slow within 5 min without changing the resting membrane potential or membrane input resistance.

B: responses digitized at a higher rate. The AHP fast, which precedes the AHP slow, is unaffected by BAPTA/AM (compare Ba with Bb). The Ca²⁺ dependence of the AHP fast is illustrated in Bc, where the neuron is superfused with 100 μM CdCl₂ for 30 s, which blocks most of the AHP fast, and all of the AHP slow. The residual component of the AHP recorded in CdCl₂ is presumably mediated by the delayed rectifier current.

C: depression of the AHP slow markedly increases neuronal excitability. The average number of APs induced by a current ramp protocol (1 nA, 2 s) increased from 1 to 5.5 Hz when the AHP slow was blocked. Scale bar represents 3 mV, 2 s in A; 15 mV, 0.25 s in B; 15 mV, 0.5 s in C.

of the neuron as evidenced by the lowering of the threshold for AP firing, and an increase in the average frequency of firing, measured during a depolarizing ramp, from 1 to 5.5 Hz (Fig. 1C).

Relation between the number of APs and the magnitude of the AHP slow current

We tested the dependence of the AHP slow on CICR by examining whether the magnitude of the AHP slow current (I_AHP) saturates with increasing numbers of APs in a manner parallel to that observed for the ΔCa₂⁺ (see Fig. 1 in Cohen et al. 1997) (and see Fig. 2). Neurons were current clamped at their resting membrane potential (approximately −60 mV) while varying numbers of APs were evoked by suprathreshold transmembrane depolarizing current pulses (2 ms, 10 Hz). One hundred milliseconds after the last AP, the neuron was voltage clamped to approximately −60 mV to measure the magnitude of the resulting I_AHP. Over the range of 1–8 APs, the amplitude of the I_AHP increased steeply. Beyond eight APs, the I_AHP amplitude began to level off, almost reaching a plateau by the 40th AP. Over the range of 1–40 APs, the relation between I_AHP amplitude and number of APs was well fit by a rectangular hyperbola (χ² = 2.35, r = 0.975, n = 10; Fig. 2B). These data are remarkably similar to those relating the amplitude of the ΔCa₂⁺ to the number of APs (dashed curve in Fig. 2B, redrawn from Cohen et al. 1997). When the I_AHP elicited by a given number of APs is plotted against the amplitude of the ΔCa₂⁺ evoked by the same number of APs, there was a close correlation (r = 0.985) between the magnitudes of ΔCa₂⁺ and I_AHP (Fig. 2C), suggesting a potential mechanistic linkage between the two.

Effects of modulators of CICR on the magnitude of the AHP slow

We have previously reported that ryanodine and SERCA inhibitors, such as thapsigargin, DBHQ, and cyclopiazonic acid (CPA) (reviewed by Inesi and Sagara 1994) can abolish caffeine- and AP-induced ΔCa₂⁺ in rabbit nodose neurons (Cohen et al. 1997), where a robust process of CICR underlies AP-induced ΔCa₂⁺. In the current experiments, we investigate the effects of these reagents on the AHP slow.

When neurons were superfused with Locke solution containing ryanodine (10 μM), thapsigargin (100 nM), DBHQ
Comparison between the time course of the AHP slow and the ΔCa

There are two mechanisms by which Ca\textsuperscript{2+} from AP-induced CICR could regulate the AHP\textsubscript{slow}. First, Ca\textsuperscript{2+} may initiate signaling cascades that ultimately control the K\textsuperscript+ channels underlying the AHP\textsubscript{slow}. Alternatively, Ca\textsuperscript{2+} itself could activate the K\textsuperscript+ channels directly, and thus exert moment-by-moment control of the AHP\textsubscript{slow}. If the AHP\textsubscript{slow} is directly dependent on Ca\textsuperscript{2+} released from the CICR pool, the AHP\textsubscript{slow} and the rise in [Ca\textsuperscript{2+}], elicited by an AP might display similar kinetics. Quantitative kinetic comparisons between these two variables are, unfortunately, subject to some uncertainty, because the time course of the ΔCa\textsubscript{A} reflects global changes in [Ca\textsuperscript{2+}], whereas the kinetics of the AHP\textsubscript{slow} are determined by events at the plasma membrane.

Nonetheless, we determined the time-to-peak and 10 to 90% decay time for both the AHP\textsubscript{slow} and the ΔCa\textsubscript{A} elicited by 1–8 APs (Table 2). The time-to-peak for ΔCa\textsubscript{A}s elicited by 1–8 APs was 1.0 ± 0.06 s (n = 32). The AHP\textsubscript{slow} evoked by 1–8 APs peaked in 1.9 ± 0.13 s (n = 25), and was significantly slower in reaching peak than the ΔCa\textsubscript{A} (P < 0.0001). The ΔCa\textsubscript{A} also decayed much more rapidly than the AHP\textsubscript{slow} (2.8 ± 0.42 s, n = 24, versus 6.7 ± 0.38 s).

**TABLE 1. Effects of CICR inhibitors on the AHP\textsubscript{slow}**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>n</th>
<th>Time to Maximum</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryanodine (10 μM)</td>
<td>5</td>
<td>15–30</td>
<td>96 ± 1.5</td>
</tr>
<tr>
<td>Thapsigargin (100 nM)</td>
<td>7</td>
<td>15–30</td>
<td>91 ± 3.7</td>
</tr>
<tr>
<td>DBHQ (10 μM)</td>
<td>9</td>
<td>6–10</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>CPA (10 μM)</td>
<td>4</td>
<td>2–5</td>
<td>93 ± 7.0</td>
</tr>
</tbody>
</table>

Values in Percent Inhibition are means ± SE and are shown as percentage change from control values; n is number of neurons. Effects of ryanodine and sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+}-ATPase inhibitors (thapsigargin, DBHQ, and CPA) on the amplitudes of slow post-spike afterhyperpolarization (AHP\textsubscript{slow}) recorded in isolated rabbit nodose neurons. The amplitudes of AHP\textsubscript{slow}, evoked by 4 action potentials (10 Hz), were recorded in normal Locke solution and in Locke solution containing DBHQ (n = 2), ryanodine (n = 2), or thapsigargin (n = 1). The average values for control ΔCa\textsubscript{A} and AHP\textsubscript{slow}, evoked by four APs, were 28 ± 4.3 nM and 7 ± 2.0 mV, respectively. Drug treatment completely abolished both the ΔCa\textsubscript{A} and the AHP\textsubscript{slow} in all five neurons. The data in Fig. 3C illustrate the effects of DBHQ on the ΔCa\textsubscript{A} and the AHP\textsubscript{slow} recorded in the same neuron. Five minutes after switching to a superfusate containing DBHQ, the ΔCa\textsubscript{A} and the AHP\textsubscript{slow} were concomitantly blocked. Parallel inhibition of the ΔCa\textsubscript{A} and the AHP\textsubscript{slow} in this neuron is illustrated in Fig. 3D. Collectively, these data support the conclusion that CICR is an important determinant of the AHP\textsubscript{slow} in rabbit nodose neurons.
These quantitative differences are graphically illustrated by Fig. 4, in which corresponding ΔCa_s and AHP_slow are scaled and overlaid. Although our preliminary observations (Cohen et al. 1994) suggested a similar time course for the AHP_slow and the ΔCa_s, quantitative analysis of a much larger data set now reveals significant temporal differences between these parameters.

DISCUSSION

Our principal finding is that Ca^{2+} release from the CICR pool is necessary for the development of the AHP_slow in nodose neurons. In virtually all rabbit nodose neurons, there exists a CICR pool that can be activated by Ca^{2+} influx resulting from a single AP (Cohen et al. 1997). By contrast, neurons with AHP_slow are randomly distributed and occur in only ~35% of the population (Fowler et al. 1985). Thus expression of the AHP_slow is dependent on a neuronal property independent of the CICR pool. The most parsimonious explanation is that the expression of the AHP_slow K+ channel determines whether a nodose neuron exhibits an AHP_slow. The recent cloning of a small conductance, Ca^{2+}-activated K+ channel (Köhler et al. 1996) with characteristics similar to the AHP_slow current may soon allow direct testing of this hypothesis.

In rabbit nodose neurons, APs induce transient increases in [Ca^{2+}], (ΔCa_s) that are dependent on an intact CICR pool (Cohen et al. 1997). If the Ca^{2+} trigger is increased by progressively increasing the number of APs from 1 to 64, Ca^{2+} influx per AP remains constant, but Ca^{2+} release from the CICR pool (i.e., the magnitude of the ΔCa) first increases sharply with the number of APs but then plateaus. This is reflected by the hyperbolic relation between the magnitude of the AP-evoked ΔCa and number of APs (dashed curve in Fig. 2B, redrawn from Cohen et al. 1997). In the current work, a similar hyperbolic relation was observed when the magnitude of I_{AHP} was plotted against a varying number of APs (Fig. 2B). Over the range of 1–16 APs, there was a close correlation (r = 0.985) between the magnitudes of the ΔCa and the I_{AHP} (Fig. 2C). This strong correlation suggests that CICR could underlie the AHP_slow.

There are several interpretations of the hyperbolic relation between the I_{AHP} and the number of APs. First, the amount of Ca^{2+} influx per AP might decrease progressively during a train of APs. However, we have demonstrated previously that over the range of 1–32 APs, the Ca^{2+} influx per AP...
TABLE 2. Time-to-peak and decay time of the AHP\textsubscript{slow} current and the Ca\textsuperscript{2+} transient evoked by varying numbers of action potentials

<table>
<thead>
<tr>
<th>Number of APs</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{\text{AHP}}) amplitude, s</td>
<td>1.3 ± 0.05 (2)</td>
<td>1.4 ± 0.12 (6)</td>
<td>1.9 ± 0.14 (9)</td>
<td>2.3 ± 0.32 (8)</td>
</tr>
<tr>
<td>(\text{Ca}^{2+}) transient</td>
<td>1.2 ± 0.05 (2)</td>
<td>0.9 ± 0.09 (7)</td>
<td>1.0 ± 0.03 (11)</td>
<td>1.2 ± 0.14 (12)</td>
</tr>
<tr>
<td>10–90% decay time, s</td>
<td>6.5 ± 3.30 (2)</td>
<td>6.8 ± 1.70 (5)</td>
<td>6.1 ± 0.50 (10)</td>
<td>6.3 ± 0.55 (7)</td>
</tr>
</tbody>
</table>

Values are means ± SE for the number of observations indicated in parentheses. Comparison of the time-to-peak and decay time of the slow post-spike hyperpolarization (AHP\textsubscript{slow}) current (\(I_{\text{AHP}}\)) and the action potential–induced \(\text{Ca}^{2+}\) transient recorded in acutely isolated nodose neurons. The time-to-peak or 10–90% decay time for \(I_{\text{AHP}}\) or \(\text{Ca}^{2+}\) transients evoked by 1–8 action potentials were not statistically different from one another (\(P > 0.05\)). The AHP\textsubscript{slow} evoked by 1–8 action potentials peaked in 1.9 ± 0.13 s and was significantly slower in reaching peak than the \(\text{Ca}^{2+}\) transient (1.0 ± 0.06 s; \(P < 0.0001\)). The \(\text{Ca}^{2+}\) transient also decayed more rapidly than the AHP\textsubscript{slow} (2.8 ± 0.42 s vs. 6.7 ± 0.38 s; \(P < 0.0001\)).

does not change (Cohen et al. 1997). Thus the plateauing of the \(I_{\text{AHP}}\)-AP relation with increasing numbers of APs must reflect another process. A second possibility is that the number of AHP\textsubscript{slow} potassium channels in nodose neurons is limiting. This too is unlikely when the concentration of extracellular \(\text{Ca}^{2+}\) is doubled, the plateau of the \(I_{\text{AHP}}\)-AP relation is elevated (unpublished observations). Finally, saturation of the \(I_{\text{AHP}}\) amplitude could reflect exhaustion of the releasable content of the CICR pool. In principle, application of caffeine, the standard CICR agonist,before and during the plateau phase could test whether the CICR pool was exhausted. Unfortunately, caffeine activates not only CICR but also a novel calcium influx pathway in these neurons (Hoesch et al. 1997) thereby complicating interpretation of such experiments.

Pharmacological evidence that supports the involvement of CICR in the generation of the AHP\textsubscript{slow} was obtained by using two functionally distinct classes of reagents to abolish CICR. Ryanodine disrupts CICR by interfering with intracellular \(\text{Ca}^{2+}\) release channels (the ryanodine receptors) (Fill and Coronado 1988). On the other hand, SERCA inhibitors (thapsigargin, DBHQ, and CPA) inhibit the ATPases that transport \(\text{Ca}^{2+}\) into stores, including the CICR pool (Inesi and Sagara 1994), thus allowing dissipation of the stores through leakage. When neurons were treated with SERCA inhibitors or with ryanodine, at concentrations and incubation times previously shown to block caffeine- and AP-induced \(\Delta\text{Ca}_{s}\) (Table 2) (Fig. 5 in Cohen et al. 1997; also unpublished observations), the AHP\textsubscript{slow} was consistently abolished (Fig. 3 and Table 1). Because these reagents do not affect \(\text{Ca}^{2+}\) influx (Cohen et al. 1997), these pharmacological data support the contention that, under physiological conditions, \(\text{Ca}^{2+}\) release from the CICR pool is necessary for development of the AHP\textsubscript{slow}. What remains unresolved, however, is whether \(\text{Ca}^{2+}\) released from the CICR pool is sufficient, by itself, to activate and sustain this persistent afterpotential. The time course of elevated \([\text{Ca}^{2+}]_{\text{i}}\), near the plasma membrane can be approximated by the duration of the \(\text{Ca}^{2+}\)-dependent fast post-spike afterpotential (AHP\textsubscript{fast}, 150 ms) that precedes the AHP\textsubscript{slow} (Fowler et al. 1985). Assuming that \(\text{Ca}^{2+}\) influx gates the AHP\textsubscript{slow} potassium channels directly (Köhler et al. 1996; Sah 1996), it seems unlikely that AP-induced \(\text{Ca}^{2+}\) influx alone could sustain this protracted afterpotential.

The significant temporal disparity between the AHP\textsubscript{slow} and the underlying \(\Delta\text{Ca}_{s}\) (Fig. 4 and Table 2) also argues against any model where \(\text{Ca}^{2+}\) ions alone are sufficient to activate the AHP\textsubscript{slow} directly. Rather, the incommensurate time courses of the AHP\textsubscript{slow} and the \(\Delta\text{Ca}_{s}\) favor a model in which activation of the AHP\textsubscript{slow} by \(\text{Ca}^{2+}\) is mediated by additional signal transduction steps. In this regard, it is notable that Lasser-Ross et al. (1997) have inferred a similar mechanism for \(\text{Ca}^{2+}\) activation of the AHP\textsubscript{slow} in vagal motoneurons, where they suggest that additional second-messenger systems may be involved. Our results and those of Lasser-Ross et al. (1997) concerning the temporal characteristics of the AHP\textsubscript{slow} and the \(\Delta\text{Ca}_{s}\) are concordant in all but

\[\text{Ca}^{2+}\]
one important respect. In vagal motoneurons, the peak of the $\Delta Ca$, coincides with the last AP. In contrast, in vagal afferent (nodose) neurons, there is a delay between the last AP and the peak of the $\Delta Ca$. This delay probably reflects the contribution of CICR to the calcium transient.

Several processes could contribute to the slow time-to-peak and long duration of the AHP$_{slow}$. These include slow diffusion of Ca$^{2+}$ ions, CICR, an enzymatic signal transduction cascade, or a combination of these factors. In CA1 pyramidal neurons of the hippocampus, there exists a well-characterized AHP$_{slow}$ with slow kinetics and physiological and pharmacological properties similar to the AHP$_{slow}$ recorded in nodose neurons (reviewed by Sah 1996). When intracellular photolabile Ca$^{2+}$ chelators (“caged” calciums) were used to rapidly increase [Ca$^{2+}$], in these neurons, the latency between Ca$^{2+}$ photorelease and the Ca$^{2+}$-induced membrane hyperpolarization was no longer than the membrane time constant (Lancaster and Zucker 1994). These results suggest that in CA1 pyramidal neurons Ca$^{2+}$ directly gates AHP$_{slow}$, K$^+$ channels and that the slow onset kinetics could arise from Ca$^{2+}$ channels residing on the proximal dendrites, distal to the AHP$_{slow}$ potassium channels that are uniformly distributed in the somata. Acutely isolated nodose neurons are essentially spherical structures lacking dendritic processes. Thus, in these neurons, the delayed onset of the AHP$_{slow}$ is unlikely to be the result of slow diffusion of Ca$^{2+}$ from distal sources. The high temperature coefficient ($Q_{10}$ $\approx$ 3.5) for the rising phase of the AHP$_{slow}$ in rabbit nodose neurons (Fowler et al. 1985) also argues against simple Ca$^{2+}$ diffusion ($Q_{10}$ $\approx$ 1.3) as an explanation for the slow kinetics (see also Sah and McLachlan 1991). One possibility is that the CICR system imposes a time dependence on the AHP$_{slow}$; another may be that opening of the AHP$_{slow}$ potassium channels requires an enzymatic signal transduction cascade. In this regard, it will be useful to learn whether rapid photorelease of Ca$^{2+}$ in nodose neurons produces an instantaneous or delayed membrane hyperpolarization.

In summary, a CICR pool in nodose neurons releases Ca$^{2+}$ in response to Ca$^{2+}$ influx evoked by single APs. One physiological role for CICR in vagal afferents is to activate and control the AHP$_{slow}$ that is responsible for spike frequency adaptation.

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